Journal of Food Protection, Vol. 61, No. 12, 1998, Pages 1615–1622

Long-Term Bacterial Profile of Refrigerated Ground Beef Made from Carcass Tissue, Experimentally Contaminated with Pathogens and Spoilage Bacteria after Hot Water, Alkaline, or Organic Acid Washes†

WARREN J. DORSA,‡ CATHERINE N. CUTTER,* AND GREGORY R. SIRAGUSA

United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, Nebraska 68933-0166, USA

MS 98-22: Received 26 January 1998/Accepted 9 July 1998

ABSTRACT

The effects of 2% (vol/vol) lactic acid (LA), 2% (vol/vol) acetic acid (AA), 12% (wt/vol) trisodium phosphate (TSP), 72°C water (HW), and 32°C water (W) washes on bacterial populations which were introduced onto beef carcass surfaces after wash treatments were determined up to 21 days of storage at 4°C of packaged ground beef prepared from the treated and inoculated carcasses. Beef carcass necks were collected from cattle immediately after harvest and subjected to the above treatments or left untreated (control). Neck meat was then inoculated with low levels (ca. <2 $\log_{10}$) of *Listeria innocua, Salmonella typhimurium, Escherichia coli* O157:H7, and *Clostridium sporogenes* contained in a bovine fecal cocktail. In general, growth of these four bacteria, aerobic bacteria, lactic acid bacteria, and pseudomonads was suppressed or not observed in the ground beef when LA, AA, or TSP treatments were used as compared to the untreated control. HW or W washes offered little suppression of growth of pathogens during subsequent storage of ground beef when these bacteria were introduced onto beef tissue posttreatment. Of the treatments used, a final LA or AA wash during the processing of beef carcasses offers the best residual efficacy for suppression of pathogen proliferation in ground beef during long-term refrigerated storage or short-term abusive temperature storage if these bacteria contaminate the carcass immediately after carcass processing.

A survey conducted by the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) determined the prevalence of some bacteria of public concern in ground beef to be 53.3% for *Clostridium perfringens,* 11.7% for *Listeria monocytogenes,* and 7.5% for *Salmonella,* and *Escherichia coli* O157:H7 was not recoverable in the 563 samples analyzed *(7).* However, studies have determined that making ground beef from carcasses containing the lowest initial bacterial populations can substantially reduce the prevalence of specific pathogenic or general bacteria *(6, 9, 16, 19, 22).* Unfortunately, few studies have considered the growth characteristics in ground beef of bacteria originating from beef carcass surface meat receiving various intervention treatments *(3, 16).* Nevertheless, the growth and survival of various pathogens common to ground beef has been thoroughly investigated using bacteria inoculated directly into the ground beef and held under refrigeration *(10, 11, 13, 17, 21, 23, 25).*

The potential for post-slaughter-process contamination by bacteria is generally accepted and has been observed *(12, 20).* Consequently, there is a need to determine the effect beef carcass interventions have on the ecology of various pathogenic bacteria when these bacteria are introduced onto the carcass following the slaughter process. The purpose of this study is to determine the microbial ecology of ground beef following experimental inoculation of beef carcasses treated with hot water, alkaline, or organic acid spray washes. Additional information will address the effects of this postprocessing contamination on the microbial ecology of ground beef following normal storage (4°C) and following exposure to temperature abuse (12°C) conditions.

MATERIALS AND METHODS

Bacteria cultures and fecal inoculum preparation. Antibiotic-resistant strains of *Escherichia coli* O157:H7 CDC B6-914, *Listeria innocua* ATCC 33090, *Salmonella typhimurium* ATCC 14028, and *Clostridium sporogenes* ATCC 11437 were isolated *(3).* Spiked bovine feces was spiral plated on sorbitol MacConkey agar with streptomycin at 250 μg/ml, Listeria selective agar with streptomycin at 500 μg/ml, Rambach agar with nalidixic acid at 250 μg/ml, and Clostridium botulinum isolation agar without egg yolk with novobiocin at 50 μg/ml to isolate and selectively enumerate the antibiotic resistant strains of *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes,* respectively. Media and component sources were described previously *(3).*

Inocula were prepared from bovine feces collected immediately postdefecation from three heifers maintained on a hay-silage diet. Ten grams of each fecal sample was combined in a sterile

* Author for correspondence. Tel: 402-762-4386; Fax: 402-762-4149; E-mail: cutter@email.marc.usda.gov.

† Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

‡ Current location: John Morrell Co., 805 E. Kemper, Cincinnati, OH 45246.

Stomacher bag (Sterifil, Spiral Biotech, Bethesda, Md.) with 270 ml of sterile physiological saline and mixed by stomaching for 1 min using a Model 400 Stomacher (Tekmar, Inc., Cincinnati, Ohio). An additional 1/10 dilution was made from this slurry.

Twenty milliliters each of *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes* grown quiescently overnight in tryptic soy broth with yeast extract (TSBYE) or in Schadler broth (Difco Laboratories, Detroit, Mich.) containing the appropriate antibiotic were pelleted by centrifugation and resuspended in 20 ml buffered peptone water. Initial culture concentrations were estimated from a McFarland turbidity standard comparison. Serial dilutions, where appropriate, were made to a final concentration of ca. 10^5 CFU/ml for each bacterium.

The final inoculum was prepared by adding 1 ml of each culture to a 15-ml sterile conical centrifuge tube, mixing by vortex, then removing 2 ml of the resulting culture mix, and adding it to 18 ml of the filtered 1/10-diluted fecal slurry described above. The final inoculum contained 3 to 4 log CFU of each marked bacterial species per ml. This inoculum, applied to tissue, resulted in levels of ca. 2 to 3 log CFU/cm^2 of *E. coli* O157:H7, *L. innocua, S. typhimurium,* and *C. sporogenes.*

Beef tissue preparation. Beef carcass necks were collected immediately after slaughter from a local cow and bull operation and transported to Roman L. Hruska U.S. Meat Animal Research Center (MARC). Three separate areas on each neck were marked with edible ink using a sterile stainless steel template and cotton swab. One area measured 10 by 10 cm (100 cm^2) and the other two measured 5 by 5 cm (25 cm^2). The beef was then subjected to intervention strategies described below. Control tissue received no treatments. After intervention treatment(s) the 100-cm^2 and 25-cm^2 surfaces were inoculated by pipetting 1 and 0.25 ml, respectively, of the inoculum onto the surface and using a sterile gloved hand to spread it over the entire marked surface. One of the 25-cm^2 areas was sampled at this point by excision as described below.

The treated and inoculated beef carcass tissue was then placed on aseptic plastic trays and stored in a segregated section of a research 4°C walk-in cooler for 24 h. After this period, 1,000 g of meat from the neck center was aseptically removed, including the marked 100-cm^2 inoculated surface, and ground. Meat was ground through a 4.5-mm head on a Model MG12, 1/4-horsepower grinder (Davpol Enterprises, Inc., New York). Nonsterile fat was added as part of the total sample to yield ca. 90% lean ground beef. The resulting ground beef was placed into a sterile stomacher bag and thoroughly mixed by hand kneading for 2 min. Fat content of the ground beef was determined using a 56.7-g sample and a fat percentage analyzer model F-100 (Needham Mfg. Co. Inc., Needham Heights, Mass.). One-hundred-gram portions were heat sealed in 3.2-mil (8-μm) nylon/copolymer bags (5 by 18 cm) with an oxygen transmission rate of 52 cm^3/m^2 at 23°C dry (Holly Sales, Omaha, Neb.), using a Hollymatic model LV 10 G (Hollymatic Corp., Countryside, Ill.), and stored at 4 or 12°C.

Wash cabinet and treatment. The wash cabinet used for this study was a stainless steel insertable pod of the commercial carcass washer (W. J. Cary Engineering, Inc., Springfield, Mo.) described by Dorsa et al. *(4).* Spray treatments were applied for 15 s at 80 lb/in^2 (5.5 bar) and 32 ± 2°C at the tissue surface, except in the case of hot water, which was sprayed at 74 ± 2°C. Pretrials with this system indicated 74°C at the nozzle would yield a maximum, consistent temperature of 70°C at the tissue surface (data not shown). All other physical parameters of the washer were set and monitored to parallel those used in previous research involving the commercial carcass washer *(4).* Wash treatments applied to the beef necks were 2% (vol/vol) DL-lactic acid (LA) (Sigma Chemical, Co., St. Louis, Mo.), 2% (vol/vol) acetic acid, glacial (AA) (Fisher Scientific, St. Louis, Mo.), 12% (wt/vol) trisodium phosphate (TSP) (Rhone-Poulenc, Cranbury, N.J.), hot water 70 ± 2°C at the tissue surface (HW), or water 32 ± 2°C at the tissue surface (W). The control group was left untreated. Each treatment was replicated six times.

Sample enumeration. A single premarked tissue section (5 by 5 cm, approximately 1 mm thick) was excised and placed into a stomacher bag for analysis immediately after treatments and inoculation. An additional tissue section (5 by 5 cm by 1 mm) was taken after refrigerated storage of the beef neck for 24 h at 4°C. Following excision, the 25-cm^2 samples were placed into a stomacher bag with 25 ml of buffered peptone water (BPW) containing 0.1% Tween 20. Additionally, an 85-g ground meat sample was taken immediately after grinding of the beef neck meat. Ground meat samples were placed into stomacher bags with 85 ml of buffered peptone water (BPW) containing 0.1% Tween 20. All meat samples were pummeled for 2 min using a Model 400 Stomacher (Tekmar, Inc.). Samples were then spiral plated using a Model D spiral plater (Spiral Systems Instruments, Bethesda, Md.) in duplicate or spread plated (1 ml total volume, over four plates) on appropriate media. All plate counts were converted to $\log_{10}$.

Mesophilic aerobic bacteria (APC), lactic acid bacteria (LAB), and pseudomonads were enumerated on Trypticase Soy Agar (TSA) (BBL Microbiology Systems, Cockeysville, Md.) at 37°C, Bacto lactobacilli agar (MRS) (BBL) with 0.02% sodium azide in 5% CO_2 at 30°C, and Pseudomonas isolation agar (PIA) (Difco Laboratories) at 37°C, respectively.

The microbial profile of the ground beef was also determined after incubation for 7, 14, and 21 days at 4°C and after incubation for 1, 2, and 3 days at 12°C as described above.

pH determination. The surface pH of the neck meat was determined using a flat surface combination probe (Corning model 245, Corning, Inc., Corning, N.Y.) immediately after treatment and after 24 h of incubation at 4°C. A spear tip probe (Corning) was used to determine the pH of ground beef immediately after grinding and after 7, 14, and 21 days of incubation at 4°C and after 1, 2, and 3 days of 12°C incubation at 12°C.

Data analysis. The average of duplicate plate counts was converted to $\log_{10}$ CFU/cm^2 or $\log_{10}$ CFU/g where appropriate. To facilitate log analysis, any plate with a 0 count was assigned a value of 10 or 1 based on the lowest limit of detection for the spiral plate or spread plate counting method, respectively. Data were analyzed by analysis of variance using the general linear model (GLM) procedure of SAS (SAS Institute, Cary, N.C.) with a probability level of 0.05 for a completely randomized design for the main effects within each storage temperature of treatment, storage time, and the interaction of these two main effects. Least squares means (LSMs) were separated with the PDIFF option.

RESULTS

The immediate effect various wash treatments had on the beef surface pH is given in Table 1. The lowest and highest pH values, observed on the tissues receiving the organic acid and alkaline treatments, respectively, persisted for 24 h. Once the tissue was ground, the average pH was 5.78 ± 0.19 regardless of the treatment received by the tissue prior to grinding. The pH for all ground meat samples dropped slightly over the 21-day study period to an average of 5.88 ± 0.18, similar to pH levels observed in this laboratory in previous studies. The average percent fat content of ground beef from beef tissue from treatment

TABLE 1. *The average pH of beef tissue surfaces and resulting ground beef from samples that were inoculated after treatment, stored at 4°C for 24 h, then converted to ground beef; ground beef samples were stored at 12°C and sampled on day 1, 2, and 3 or stored at 4°C and sampled on day 7, 14, and 21*

	Treatment[a]					
Sample period (days)	C	W	HW	2.0% LA	2.0% AA	12% TSP
<0.5 h[b]	8.34	8.17	7.84	4.18	4.49	10.94
24 h	7.49	7.83	8.27	4.46	4.57	9.23
0[c]	5.97	5.73	5.68	5.82	5.66	5.85
1	5.99	5.76	5.77	5.80	5.73	5.89
2	6.01	5.76	5.79	5.84	5.77	5.93
3	5.96	5.71	5.72	5.78	5.72	5.87
7	6.06	5.82	5.84	5.89	5.81	5.97
14	5.98	5.74	5.76	5.81	5.74	5.91
21	6.06	5.76	5.81	5.88	5.78	6.00

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] pH values were taken from beef surface tissue immediately after treatment (<0.5 h) and after 24 h at 4°C.
[c] pH values were taken from resulting ground beef samples on days 0; 1, 2, 3 (12°C); 7, 14, 21 (4°C).

groups W, HW, LA, AA, TSP, and C (untreated) was 10.9, 10.7, 10.8, 10.4, 10.5, and 10.9, respectively. Excessive gas formation (blowup) was observed at the 21-day sample period for one of six study replications in packs containing ground beef incubated at 4°C and receiving no treatment. No other blowups were observed throughout the study period.

Analysis of APC populations did not demonstrate a significant difference between untreated control or W- and HW-treated samples immediately after inoculation of the beef tissue samples following the wash treatment (Table 2). LA-, AA-, and TSP-treated beef tissue samples had significantly lower APC populations than the untreated control. By the end of the 3-day period of storage at 12°C there was as much as a 1.5-log difference between TSP-treated samples and the untreated control; however, this was not significantly different. By the end of the 21-day period of storage at 4°C samples receiving any treatment had considerably lower APC populations than the untreated C samples, but only HW- and LA-treated samples were significantly lower. Storage time was determined to be a significant effect for APC for samples held at 4 or 12°C, but the interaction of treatment and storage was not.

When analyses of treatment were determined for specific storage times and temperatures, LAB populations were found to be significantly lower than the controls on any antimicrobial-treated beef surface tissue immediately after inoculation and remained low for the 24-h period of incubation at 4°C (Table 3). Once the tissue was ground, antimicrobial activity was lost, as indicated by lack of significant difference between any treated tissue and C. After 3 days of storage at 12°C a lack of significant difference between all samples was observed. This was also true for samples incubated at 4°C for 21 days with the exception of AA-treated samples, which had significantly lower LAB populations than the C samples. Storage time was determined to be a significant effect for LAB for samples held at 4 or 12°C, but the interaction of treatment and storage was not.

Treatments were determined to be a significant effect for pseudomonad populations for samples held at 4 or 12°C, but storage times and the interaction of the two main effects was not. When treatment effect was determined for specific storage times and temperatures, pseudomonads were not detectable on HW-, LA-, or AA-treated samples immediately after inoculation and the pseudomonad populations were significantly lower than in the C samples (Table 4). After grinding, populations from HW and LA samples remained below detectable limits. After 21 days of storage at 4°C pseudomonads were not detected in any treated samples while exhibiting a mean population value of 1.0 $\log_{10}$ CFU/g in the C samples. Pseudomonads were detected in all sample

TABLE 2. *The least squares means of mesophilic aerobic bacteria (APC populations) on beef tissue surfaces ($\log_{10}$ CFU/cm^2) and in resulting ground beef ($\log_{10}$ CFU/g)*

		Treatment[a]					
Sample period[b]	Storage temp.	C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.24)[c]	—	3.8A[d]	3.4AB	3.3AB	2.7BC	3.0BC	2.5C
B (0.30)	4.0°C	3.4A	3.8A	3.6A	2.7AB	3.0AB	2.4B
0 (0.17)	—	2.6A	2.7A	2.6A	2.3AB	2.2AB	1.9B
1 (0.21)	12.0°C	2.8A	2.7A	2.6A	2.3A	2.4A	2.3A
2 (0.37)	12.0°C	3.4A	2.8AB	2.8AB	2.6AB	2.2B	2.0B
3 (0.61)	12.0°C	4.2A	3.6A	3.5A	3.4A	3.2A	2.7A
7 (0.36)	4.0°C	3.3A	2.6AB	2.5AB	2.1B	2.5AB	1.9B
14 (0.60)	4.0°C	5.2A	3.9AB	3.1B	3.1B	3.5B	3.3B
21 (0.88)	4.0°C	6.0A	4.3AB	3.0B	3.1B	4.8AB	3.9AB

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

TABLE 3. *The least squares means of lactic acid bacteria (LAB populations) on beef tissue surfaces (log_{10} CFU/cm²) and in resulting ground beef (log_{10} CFU/g)*

Sample period[b]	Storage temp.	Treatment[a] C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.16)[c]	—	2.1A[d]	1.6B	1.7AB	0.1C	0.5C	0.2C
B (0.16)	4.0°C	1.1B	1.8A	0.8B	0.1C	0.2C	0.1C
0 (0.13)	—	0.2ABC	0.5A	0.4AB	0.3AB	0.1BC	0.0C
1 (0.25)	12.0°C	0.8A	0.8A	0.5A	0.7A	0.4A	0.1A
2 (0.45)	12.0°C	2.3A	1.6AB	1.2B	1.6AB	1.2B	0.9B
3 (0.64)	12.0°C	2.8A	2.6A	1.5A	2.4A	2.2A	1.6A
7 (0.40)	4.0°C	1.7A	0.6AB	0.6AB	0.5B	0.8AB	0.0B
14 (0.81)	4.0°C	2.8A	1.9AB	0.4B	2.1AB	1.1AB	0.4B
21 (0.89)	4.0°C	3.9A	2.9AB	1.5B	2.2AB	1.3B	2.0AB

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

types after 3 days at 12°C, but these population levels were significantly lower in the C samples than for HW-, LA-, and AA-treated samples.

E. coli O157:H7 was not detected on LA- or TSP-treated samples immediately after inoculation onto beef tissue and was detected at significantly higher levels in the C samples (Table 5). After grinding, there was no significant difference between populations from HW, LA, AA, TSP, and C samples. The bacterium remained undetected in the LA- and TSP-treated samples. After 3 days at 12°C *E. coli* O157:H7 was not detected in any of the antimicrobial-treated samples, and the populations of this organism in W, HW, LA, AA, and TSP samples were significantly lower than in C samples. When samples were held at 4°C for 21 days, *E. coli* O157:H7 was not detected in any samples except for some replications of HW-treated samples; however, the population levels in the latter samples were low, and there were no significant differences observed between any of the mean values. Storage times and the interaction of treatment and storage time were determined to be a significant effect for *E. coli* O157:H7 held at 12°C. While treatments were determined to be a significant effect at 4°C, the storage times ($P = 0.069$) and the interaction of the two main effects ($P = 0.047$) were not.

L. innocua was present at significantly higher levels on W, HW, and C samples than LA, AA, or TSP samples immediately after inoculation (Table 6). After grinding, the bacterium was not detected in any of the samples that had received a chemical antimicrobial treatment, while present in W, HW, and C samples. This condition remained consistent throughout both 4 and 12°C storage, except in the case of TSP, where very low levels were detected after 21 days at 4°C. After both storage periods W and HW samples exhibited significantly lower population means than the C

TABLE 4. *The least squares means of pseudomonads on beef tissue surfaces (log_{10} CFU/cm²) and in resulting ground beef (log_{10} CFU/g)*

Sample period[b]	Storage temp.	Treatment[a] C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.17)[c]	—	0.5AB[d]	0.6A	0.0C	0.0C	0.0C	0.1BC
B (0.10)	4.0°C	0.1B	1.3A	0.0B	0.0B	0.0B	0.0B
0 (0.12)	—	0.2AB	0.5A	0.0B	0.0B	0.2AB	0.1B
1 (0.19)	12.0°C	0.5A	0.5A	0.2A	0.2A	0.1A	0.0A
2 (0.30)	12.0°C	0.9A	0.1B	0.1B	0.0B	0.2B	0.4AB
3 (0.54)	12.0°C	2.0A	0.6AB	0.1B	0.1B	0.1B	0.6AB
7 (0.18)	4.0°C	0.6A	0.0B	0.0B	0.0B	0.1AB	0.0B
14 (0.55)	4.0°C	1.5A	0.0B	0.0B	0.0B	0.0B	0.9AB
21 (0.41)	4.0°C	1.0A	0.0A	0.0A	0.0A	0.0A	0.0A

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

TABLE 5. *The least squares means of* Escherichia coli *O157:H7 on beef tissue surfaces (log_{10} CFU/cm²) and in resulting ground beef (log_{10} CFU/g)*

Sample period[b]	Storage temp.	Treatment[a] C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.11)[c]	—	2.1A[d]	1.8AB	1.9AB	0.0C	1.6B	0.0C
B (0.11)	4.0°C	1.4B	1.8A	1.6AB	0.0C	0.2C	0.0C
0 (0.08)	—	0.1B	0.4A	0.1B	0.0B	0.1B	0.0B
1 (0.11)	12.0°C	0.3AB	0.6A	0.1BC	0.0C	0.1BC	0.0C
2 (0.22)	12.0°C	1.3A	0.2B	0.3B	0.0B	0.1B	0.0B
3 (0.32)	12.0°C	1.9A	0.5B	0.8B	0.0B	0.0B	0.0B
7 (0.05)	4.0°C	0.1A	0.2A	0.0A	0.0A	0.1A	0.0A
14 (0.00)	4.0°C	0.0A	0.0A	0.0A	0.0A	0.0A	0.0A
21 (0.09)	4.0°C	0.0A	0.0A	0.2A	0.0A	0.0A	0.0A

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

samples, but significantly higher levels than found in any of the chemical antimicrobial-treated samples. Storage times and the interaction of treatment and storage time were determined to be a significant effect for *L. innocua* held at 4 or 12°C.

Treatments were determined to be a significant effect for *S. typhimurium* held at 4 or 12°C (Table 7). *S. typhimurium* was not detected on LA- or TSP-treated samples of beef carcass surface tissue after inoculation. The bacterium remained undetectable after the beef was ground for these two treatments. However, when treatment effect was determined for specific storage times and temperatures, there was no statistical difference between the control mean and any of the sample means, regardless of treatment. After 21 days of 4°C storage, the LA- and TSP-treated samples remained below the detectable limits, but all sample means remained statistically similar. After 3 days at 12°C the chemical antimicrobial treatment samples yielded significantly lower levels of *S. typhimurium* than the W, HW, and C samples, as much as 3.1 log_{10} lower than C samples. For incubation at 12°C storage time was determined to be significant, but this was not the case for incubation at 4°C or for either storage temperature for the interactions.

Treatments, storage times, and the interaction were determined to be significant effects for *C. sporogenes* held at 4°C (Table 8). At 12°C only treatments were determined to produce a significant effect. Vegetative cells of *C. sporogenes* were not detected in any samples receiving an antimicrobial treatment at any time during the study. The C samples did exhibit the presence of the bacterium throughout the 12°C incubation, but it was not detected after 14 days when samples were incubated at 4°C. The W- and HW-treated samples behaved similarly to the C samples.

TABLE 6. *The least squares means of* Listeria innocua *on beef tissue surfaces (log_{10} CFU/cm²) and in resulting ground beef (log_{10} CFU/g)*

Sample period[b]	Storage temp.	Treatment[a] C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.15)[c]	—	1.9A[d]	1.5A	1.5A	0.1B	0.3B	0.0B
B (0.19)	4.0°C	1.4A	1.9A	1.4A	0.0B	0.0B	0.0B
0 (0.12)	—	0.3BC	0.9A	0.7AB	0.0C	0.0C	0.0C
1 (0.15)	12.0°C	1.0A	0.9A	0.7A	0.0B	0.0B	0.0B
2 (0.25)	12.0°C	1.9A	1.1B	0.7B	0.0C	0.0C	0.0C
3 (0.35)	12.0°C	2.7A	1.5B	1.2B	0.0C	0.0C	0.0C
7 (0.25)	4.0°C	2.0A	0.9B	0.7BC	0.0C	0.0C	0.0C
14 (0.46)	4.0°C	3.3A	1.4B	1.2BC	0.0C	0.0C	0.0C
21 (0.57)	4.0°C	4.2A	2.0B	1.9BC	0.0C	0.0C	0.3C

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

TABLE 7. *The least squares means of* Salmonella typhimurium *on beef tissue surfaces (log_{10} CFU/cm²) and in resulting ground beef (log_{10} CFU/g)*

Sample period[b]	Storage temp.	Treatment[a] C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.24)[c]	—	2.9A[d]	2.6A	2.8A	0.0B	2.4A	0.0B
B (0.39)	4.0°C	2.0AB	2.8A	2.6A	0.1C	1.1B	0.1C
0 (0.18)	—	0.5A	0.5A	0.5A	0.0A	0.1A	0.0A
1 (0.46)	12.0°C	1.3A	1.4A	1.3A	0.0B	0.4AB	0.0B
2 (0.54)	12.0°C	2.4A	1.6AB	1.8AB	0.0C	0.4BC	0.0C
3 (0.59)	12.0°C	3.2A	2.3A	2.4A	0.2B	0.5B	0.1B
7 (0.23)	4.0°C	0.5AB	0.9A	0.7A	0.0B	0.3AB	0.0B
14 (0.17)	4.0°C	0.3AB	0.5A	0.3AB	0.0B	0.2AB	0.0B
21 (0.13)	4.0°C	0.3A	0.3A	0.1A	0.0A	0.2A	0.0A

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

DISCUSSION

The initial APC observed on the beef surface after treatment and inoculation with feces was similar to that observed in a previous study in this laboratory *(5)*. Antimicrobial treatments were effective for reducing APC populations on beef surface tissue to a significantly lower level than on controls (Table 2). This effect was, for the most part, transferred into the ground samples and remained somewhat observable after 3 days at 12°C; however, at that time there was no significant difference between any sample types. The long-term effectiveness was more easily observed for antimicrobially treated ground beef when it was stored at 4°C for up to 21 days. Regardless of the incubation temperature, it should be noted that the control mean was considerably higher than the mean for any treated samples at the end of the study. While the lower counts for chemical antimicrobial-treated samples were expected, the lower counts for HW samples were not. Organic acids have been shown to afford some residual antimicrobial effect on beef surface tissue and also in the resulting ground beef during other studies *(3–5, 24)*. However, HW treatments should not afford the surface any residual antimicrobial capabilities and this type of effect has not been observed during other studies in this laboratory *(3–5)*. The effect was also observed for LAB populations in HW-treated samples (Table 3). Since LAB have been determined to be the prevalent microflora during extended refrigeration of ground beef *(8, 14)*, the low counts observed for APC are likely a reflection of reduced LAB levels.

Pseudomonads counts were generally lower than in a similar study conducted in this laboratory *(3)*. However, the overall patterns of growth and survival experienced for pretreatment-inoculated pseudomonads observed previously

TABLE 8. *The least squares means of* Clostridium sporogenes *on beef tissue surfaces (log_{10} CFU/cm²) and in resulting ground beef (log_{10} CFU/g)*

Sample period[b]	Storage temp.	Treatment[a] C	W	HW	2.0% LA	2.0% AA	12% TSP
A (0.29)[c]	—	2.3A[d]	1.7A	1.7A	0.0B	0.0B	0.0B
B (0.13)	4.0°C	0.3A	0.3A	0.0A	0.0A	0.0A	0.0A
0 (0.17)	—	0.6A	0.6A	0.3AB	0.0B	0.0B	0.0B
1 (0.10)	12.0°C	0.4A	0.1AB	0.1AB	0.0B	0.0B	0.0B
2 (0.09)	12.0°C	0.4A	0.1B	0.0B	0.0B	0.0B	0.0B
3 (0.10)	12.0°C	0.3A	0.1AB	0.0B	0.0B	0.0B	0.0B
7 (0.07)	4.0°C	0.2A	0.0A	0.0A	0.0A	0.0A	0.0A
14 (0.00)	4.0°C	0.0A	0.0A	0.0A	0.0A	0.0A	0.0A
21 (0.00)	4.0°C	0.0A	0.0A	0.0A	0.0A	0.0A	0.0A

[a] C, control; W, water, 32°C; HW, hot water, 70°C; LA, lactic acid; AA, acetic acid; TSP, trisodium phosphate.
[b] Bacterial counts were taken from beef surface tissue immediately after inoculation after receiving treatments (A) and after a period of 24 h at 4°C (B) and from the resulting ground beef immediately after preparation (0) and after various storage periods indicated in days.
[c] Values in parentheses indicate the pooled standard error of the mean for each row.
[d] Means within a row with no common following letters are significantly different ($P \leq 0.05$).

were not changed. Low initial numbers of pseudomonads were observed on the W and C samples, but pseudomonads were not detected on the initial HW, LA, AA, or TSP samples or in the HW, LA, AA, and TSP samples after grinding or at the end of the 21 days at 4°C. While some growth was detected in these samples after 3 days at 12°C, the levels in HW-, LA-, and AA-treated samples were significantly lower than those in the C samples. Based on these results it would appear that organic acid or HW carcass treatments will aid in the extension of the time before the onset of spoilage should the resulting ground beef be subjected to mild thermal abuse.

An attempt to introduce detectable levels of *E. coli* O157:H7 into ground beef through post-slaughter-process contamination of the carcass with low levels of the bacterium was effectively controlled by the LA and TSP treatments. As observed in a previous study *(5)*, AA treatments were less effective than LA and TSP at producing initial reductions, but by the end of the storage periods the bacterium was undetected. After 21 days at 4°C, *E. coli* O157:H7 was not detected except in HW-treated samples at low levels. However, at the warmer storage temperature of 12°C, the C samples had a significantly higher mean value than any treated samples. As was determined for beef carcass surface tissue *(5)*, LA, AA, and TSP carcass washes generally provided the best suppression of *E. coli* O157:H7 in ground beef as evidenced by the inability to detect it after 3 days when the ground beef was stored at 12°C.

All chemical antimicrobial treatments effectively reduced the levels of *L. innocua* on the subsequently inoculated beef surface tissue in a similar fashion to that observed in other studies conducted in this laboratory *(5)*. Once the tissue was ground, the bacterium was not detectable, and it remained that way throughout the study. TSP-treated samples, while not significantly different from the organic acid treatments, did allow *L. innocua* to recover to detectable levels after prolonged storage at 4°C. The reduced ability of TSP to control the growth of *L. innocua* for extended periods of time on beef surface tissue has been previously documented *(4, 5)*.

L. innocua did not grow well in ground beef resulting from tissues previously subjected to W or HW treatments. This was not unexpected since other studies have demonstrated that *L. monocytogenes* does not grow well in ground beef when held under refrigerated temperatures for up to 15 days *(2, 13, 15)*. However, *L. innocua* grew more rapidly in C samples, achieving a mean level of 4.2 $\log_{10}$ CFU/g after 21 days of refrigerated storage. The ability of the bacterium to grow to significantly greater levels in C samples compared to W- or HW-treated samples has not been observed in previous studies involving similar treatments in this laboratory *(3)* and seems to contradict observations made by other researchers *(2, 13, 15)*. This finding would support that of Barbosa et al. *(1)*, who observed that *L. monocytogenes* grew more rapidly in ground beef with a pH above 6.0. The 21-day pH of the ground beef in the current study was 6.06, unlike past studies conducted in this laboratory when the pH of untreated control samples held under similar temperatures and times was determined to be 5.59 *(3)*. During the present study the pH values of C samples (6.06) were determined to be significantly higher ($P < 0.001$) than that of the W- or HW-treated samples (pH 5.76 and 5.81, respectively). Since the final pH of ground beef is somewhat variable, it appears that of the interventions studied the use of an organic acid or TSP carcass wash intervention would add the most significant level of protection from post-carcass-process contamination by *Listeria* spp. for ground beef.

As with the two previous bacteria discussed, AA treatments seemed to produce the smallest initial reductions in *S. typhimurium* populations when compared with LA or TSP. However, by the end of the 12°C storage period, the LA, AA, and TSP samples all had means significantly lower than the washed samples or the C samples. When samples were held for 21 days at 4°C, *S. typhimurium* levels did not change substantially in any of the sample types over time. This was expected since it is well documented that *Salmonella* spp. do not grow well at refrigerated temperatures *(18)*. However, the occurrence of *Salmonella* spp. in retail ground beef has been documented through survey studies *(7, 19)*. Mates *(19)* determined that the incidence of *Salmonella* contamination in 519 samples of frozen ground beef paralleled the increase in aerobic bacterial counts and probably reflected poor sanitation during handling. The current study indicates the use of a chemical antimicrobial wash during beef carcass processing would substantially reduce the risk of *Salmonella* presence in the resulting ground beef.

Populations of *C. sporogenes* introduced posttreatment responded in this study to antimicrobial treatments in a way similar to that in previous studies on beef surface tissue *(5)*. The gradual reduction over time of different *Clostridium* spp. inoculated into ground beef and stored at various refrigeration temperatures has been observed by other researchers *(10)*. The persistence of the bacterium in the C samples and nondetection from antimicrobial-treated ground beef after short-term storage at 12°C demonstrate the value of using an antimicrobial on beef carcasses during the slaughter process.

In general, this study demonstrates the benefits of a decontamination step, as a last step during the slaughter process for beef carcasses, to the resulting ground beef. In particular, the initial carcass surface bacterial reductions produced by chemical antimicrobial spray applications are observable in the ground beef produced from these carcasses and reductions are maintained over time during refrigerated storage. Additionally, chemical antimicrobial carcass sprays tend to minimize the positive effect which short-term temperature abuse has on pathogen growth and survival in ground beef.

ACKNOWLEDGMENTS

The authors wish to thank Dawn Wiseman, Julie Dyer, Carole Smith, and Jane Long for their expert technical assistance, and also James Wray and Darrell Light for statistical analyses.

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